

REMARKS

At the outset, applicants would like to thank Examiner Davis for her time and consideration of the present application at the interview with the undersigned attorney. At the interview, the issues raised in the outstanding Official Action were discussed.

Claims 56-75 are pending in the present application. Claims 39-55 have been canceled. Support for claims 56-75 can be found in previously pending claims 19-38 and generally throughout the specification. In particular, support for claims 56-75 may be found in the present application at pg. 6, lines 10-20 and page 7, lines 10-20.

New claim 56 corresponds with previous claim 39, with the proviso that the long chain polyunsaturated fatty acid ratio is no longer recited. Rather, dependent claims 58 and 61 recite the claimed ratios previously recited in claim 39.

After reviewing the present application, applicants believe that the publications, alone or in combination with each other, do not teach the claimed invention. Indeed, applicants note that none of the proposed combination of references direct one skilled in the art to combine and modify their respective teachings in a manner so as to obtain the claimed invention. Indeed, applicants submit that none of the proposed combination of references result in a preparation that exhibits the unexpected results exhibited by the claimed range. At this time,

the Examiner's attention is respectfully directed to the attached declaration by Martijn C. de Wilde. Applicants believe that the declaration teaches that the claimed preparation of claim 1 provides an unexpected and improved benefit over previous preparations. Applicants believe that the experiments set forth in the declaration show that the presently claimed combination of long chain polyunsaturated fatty acids (PUFA), methionine metabolism factors and phospholipids (see supplement 2) exhibit unexpected and improved blood vessel health and improved spatial memory.

As noted in the declaration by Martijn C. de Wilde, a 2-vessel occlusion method, a method commonly employed in studies on aging and dementia, is used to compare the effects of the claimed preparation relative to a control diet. Upon reviewing the results, applicants believe that it is clear that essential fatty acids (EFAs) or EFAs together with polyunsaturated fatty acids (PUFAs) do not restore vascular function in atherosclerosis animal models. Rather, the combination of polyunsaturated fatty acids, vitamins and phospholipids according to the claimed invention, provides markedly improved unexpected benefits over a control diet.

Upon reviewing the Official Action of May 17, 2004, applicants do not believe that any of the proposed combinations of publications renders obvious the claimed invention.

Claims 39, 40, 42, 44, 48, and 51 were rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN 4,810,497, DELLA VALLE et al. 4,595,680 and FUGH-BERMAN et al. This rejection is respectfully traversed.

Applicants believe that the proposed combination of HORROBIN, DELLA VALLE et al. and FUGH-BERMAN et al. fails to disclose or suggest the claimed invention. HORROBIN describes a long-chain fatty acid composition supplying 20 mg to 10 g of a total amount of EPA + DHA + D(H)GLA + AA relative to the total amount of 2-30 g of LA + ALA (see column 2, lines 55-59).

In an effort to remedy the deficiencies of HORROBIN, the outstanding Official Action cites to DELLA VALLE et al. However, DELLA VALLE et al. do not teach the claimed combination or amounts of long-chain fatty acids, phospholipids or methionine metabolism factors.

In a further effort to remedy the deficiencies of HORROBIN and DELLA VALLE et al., the Official Action cites to FUGH-BERMAN et al. FUGH-BERMAN et al. teach that DHA and EPA, but not AA and DHGLA, may be added to a dietary supplement. However, at best, the teachings of FUGH-BERMAN et al. are speculative in nature. Indeed, FUGH-BERMAN et al. teach that more research should be conducted before natural products can be administered as psychotherapeutic agents (abstract). Moreover, applicants note that FUGH-BERMAN et al. fail to teach or even mention the claimed Ω -3/ Ω -6 ratio recited in claim 61.

As a result, applicants believe that the proposed combination of HORROBIN, DELLA VALLE et al. and FUGH-BERMAN et al. fails to teach the claimed invention. Indeed, applicants respectfully submit that none of the publications, alone or in combination, provide the motivation or reasonable expectation of success to one skilled in the art to combine and modify their respective teachings to obtain the claimed invention.

The Examiner is respectfully reminded that a particular parameter must first be recognized as a result-effective variable, i.e., a variable which achieves a recognized result, before the determination of the optimum or workable ranges of the variable might be characterized as routine experimentation. *In re Antonie* 559, F.2d 618, 195 USPQ 6 (CCPA 1977). None of the publications teach or suggest the claimed combination of components. Moreover, the proposed combination of references fails to disclose or suggest any of the claimed ratios recited in the dependent claims.

Indeed, applicants note that the outstanding Official Action acknowledges on page 5 that HORROBIN and DELLA VALLE et al. actually teach that the disclosed fatty acids and phospholipids should be added in specific amounts to their respective compositions. As a result, applicants believe that the cited publications fail to show that any of the claimed amounts and ratios are simply a matter of routine experimentation. In fact, the cited publications actually teach

distinctly lower amounts than those claimed in the present application.

As a result, applicants submit that the Official Action fails to show that one of ordinary skill in the art would consider the claimed amounts and ratios as result-effective variables.

Thus, in view of the above, applicants believe that the proposed combination of HORROBIN, DELLA VALLE et al. and FUGH-BERMAN et al. fails to render obvious the claimed invention.

Claims 52, 53 and 55 were rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and TAIYO FISHERY CO., LTD. This rejection is respectfully traversed.

Applicants respectfully submit that the TAIYO FISHERY CO., LTD. publication fails to remedy the deficiencies of HORROBIN, DELLA VALLE et al. and FUGH-BERMAN et al. Indeed, the TAIYO FISHERY CO., LTD. publication does not even discuss long-chain fatty acids and would not lead one skilled in the art to combine and modify the respective teachings of each publication to obtain the claimed invention.

Thus, applicants believe that the proposed combination fails to render obvious the claimed invention.

Claims 41 was rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al.,

FUGH-BERMAN et al. and YU et al. 5,177,082. This rejection is respectfully traversed.

YU et al. are silent as to the claimed combination of components recited in the present application. As a result, applicants believe that YU et al. fail to remedy the deficiencies of the above-identified publications.

Claims 43, 44, and 54 were rejected under 35 USC 103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and SMITH et al. 6,008,221. This rejection is respectfully traversed.

Upon reviewing SMITH et al., SMITH et al. neither disclose nor suggest the amounts and ratios as set forth in the claimed invention. Thus, applicants believe that the proposed combination fails to render obvious claims 56-75.

Claims 44 and 45 were rejected under 35 USC 103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and HUTTERER 4,837,219. This rejection is respectfully traversed.

HUTTERER also fails to disclose or suggest the claimed combination of components and ratios. Thus, applicants believe that the proposed combination of HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and HUTTERER does not teach the claimed invention.

Claim 50 was rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN

et al., SMITH et al., HUTTERER and GLICK 5,004,615. This rejection is respectfully traversed.

GLICK discusses the administration of dietary supplements for preventing and controlling dementia and memory loss. However, GLICK fails to disclose or suggest the administration of a preparation having the claimed combination of components and ratios as recited in the present application.

As a result, applicants believe that the proposed combination fails to render obvious the claimed invention.

Claims 46-47 were rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and RABIEN (DE 4309217). This rejection is respectfully traversed.

The Official Action of May 17, 2004 states that RABIEN teaches a composition comprising alpha lipoic, pantothenic acid and vitamin E for treating Alzheimer's disease. However, RABIEN does not provide any motivation to one skilled in the art to combine and modify the proposed combination of publications in a manner so to obtain the claimed invention. As a result, applicants believe that RABIEN fails to remedy the deficiencies of the above-identified publications and request that the rejection be withdrawn.

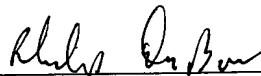
In view of the present amendment and the foregoing remarks, therefore, it is believed that this application is now in condition for allowance with claims 56-75, as presented.

Allowance and passage to issue on that basis are accordingly respectfully requested.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. §1.16 or under 37 C.F.R. §1.17.

Respectfully submitted,

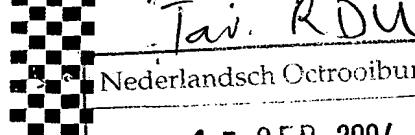
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Appendix:

Declaration under Rule 132 by Martijn C. de Wilde



Tai. RDU

Nederlandsch Oftrooibureau

15 SEP 2004

INGELOPEN:
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Amanda Johanne Kiliaan et al.

Serial No.: 09/703,798

Filed: November 2, 2001, Continuation-in-part of May 2, 2000 (09/566, 386)

Title: PREPARATION FOR THE PREVENTION AND/OR TREATMENT
OF VASCUALR DISORDERS

Conf: 2164

Group: 1651

Examiner: Ruth A. Davis

September 15, 2004

DECLARATION UNDER RULE 132

Assistant Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Martijn C. de Wilde, residing at Pomona 260, 6708 CJ Wageningen, The Netherlands, do hereby declare that:

1. I am a citizen of the Netherlands,
2. My educational and technical background in the field of Biology is as follows:

a) I am a M.Sc. graduate from the Department of Molecular Neurobiology, Groningen University, Groningen, The Netherlands, specialisation Behavior and Neuroscience in 1999 (with distinction);

b) From 2000 till 2002, I was employed at the Department of Animal Physiology, Groningen University, Groningen, The Netherlands

c) I have been employed by Nutricia N.V. since 2002, presently as a junior scientist dealing with research and the process design of behavioral testing and evaluation of nutritional interventions in animal models for neurodegenerative disease

3. I have read Kiliaan et al. US application 09/703,798 filed November 2, 2001;
4. I make this declaration in support of the present application, and to provide evidence demonstrating that one of ordinary skill in the art would not find the presently-claimed invention obvious in view of several publications cited in the Office Action mailed on May 17, 2004.

In the Office Action, claims 39, 40, 42, 44, 48, 48 and 51 were rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN 4,810,497, DELLA VALLE et al. 4,595,680 and FUGH-BERMAN et al.

Claims 52, 53 and 55 were rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and TAIYO FISHERY CO., LTD.

Claim 41 was rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and YU et al. 5,177,082.

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Claim 50 was rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al., SMITH et al., HUTTERER and GLICK 5,004,615.

Claims 46-47 were rejected under 35 USC §103(a) as allegedly being unpatentable over HORROBIN, DELLA VALLE et al., FUGH-BERMAN et al. and RABIEN (DE 4309217).

After reviewing the Office Action, I do not believe that any of the proposed combinations of publications disclose or suggest the claimed invention. The

publications, alone or in combination with each other, simply do not teach the recited combination of components or ratios in the pending claims. In addition, I do not believe that any of the proposed combination of references would lead to the unexpected results exhibited by the claimed invention shown in the experimental data discussed below:

EXPERIMENTAL DATA

Introduction

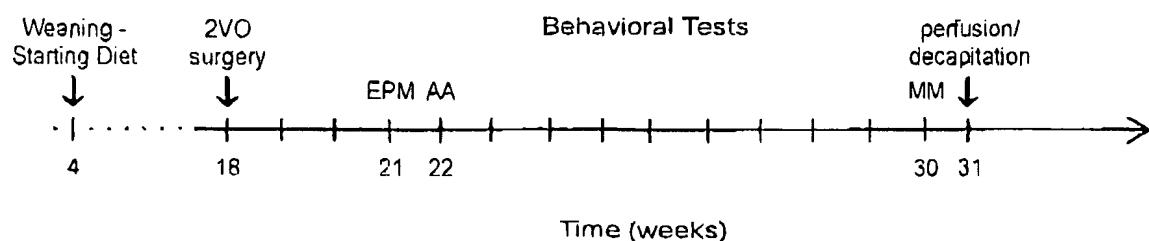
A two-vessel occlusion (2VO) method in rats leads to reduced blood flow to the brain, comparable to that seen in atherosclerosis. This condition is a major risk factor for the development of dementia, in particular vascular dementia and Alzheimer's disease (Clarke et al, Arch Neurol 1998 55: 1449-1455; Miller et al, Nutrition 2000 16:675-677).

The 2VO method is common practice in studies on aging and dementia (see e.g. E. Farkas, P.G. Luiten "Cerebral microvascular pathology in aging and Alzheimer's disease" Prog. Neurobiol. 64 (2001) p. 575-611; G.I. de Jong, E. Farkas, C.M. Stienstra, J.R. Plass, J.N. Keijser, J.C. de la Torre, P.G. Luiten "Cerebral hypoperfusion yields capillary damage in the hippocampal CA1 area that correlates with spatial memory impairment" Neuroscience 91 (1999) p. 203-210).

In this model, the effects of 2VO as well as of two experimental supplements, of one control diet (placebo) and of the interaction between 2VO and the supplements were tested on parameters of blood vessel health, spatial learning performance and neuroendocrine output (circulating corticosterone levels).

Experimental design

A scheme of the experiment in time is shown below. The abbreviations will be explained in the text.



Animals

Sixty, 30-day old male Wistar rats were randomly assigned to three groups receiving different diets (20 animals per group). The animals were socially housed in cages of five throughout the experiments. Food and water were available ad libitum. The weight of the animals was checked weekly.

Diets

The three diets were identical with respect to the composition and amount of carbohydrates, proteins, minerals and caloric value, and were manufactured in the form of regular food pellets. The main characteristics in accordance with the invention are listed below:

- control or placebo diet contains soy oil-based essential fatty acids EFA's (LA, ALA) and essential vitamins (folate, B6, B12, E, Se and choline);
- supplement 1 contains polyunsaturated fatty acids PUFA's (EPA, DHA, AA) and extra vitamins/antioxidants (B12, E, C, Se) in addition to the components of the control diet; and
- supplement 2 contains PUFA's (EPA, DHA, AA) and extra vitamins/antioxidants (B12, E, C, Se) according to supplement 1, and structural phospholipids PL's (PC, PS and choline) additionally.

The control or placebo diet contains EFA's and specific vitamins and minerals that completely meet the animals nutritional dietary requirements (Nat Res Council, 4th ed, 1995). The composition of the placebo diet can be considered optimal and is not deficient with respect to any essential nutritional component and enriched with respect to vitamins, antioxidants and EFA's compared to levels following normal intake in humans in the western society.

In table 1 the individual ingredients of the three diets/supplements are listed, together with the amount thereof in 100 g of the total food composition. The supplements 1 and 2 are given in addition to the control diet, meaning that all food constituents in the control diet are also present in the supplements 1 and 2.

Supplement 1 corresponds to the composition taught in Horrobin (US 4,810,497), said composition comprising long chain polyunsaturated fatty acids, especially DGLA, AA, EPA, DHA, GLA, linoleic acid and α -linolenic acid, and citrate.

Supplement 2 is in accordance with the invention, the preparation comprising a fraction comprising long chain polyunsaturated fatty acids, a fraction comprising phospholipids and a fraction comprising a methionine metabolism factor selected from the group consisting of folic acid, vitamin B12, vitamin B6, magnesium and zinc.

Table 1: List of ingredients in the different diets/supplements

	Control diet (in g/100 gram food)	Supplement 1 (in g/100 gram food)	Supplement 2 (in g/100 gram food)
<i>Methionine factor:</i>			
Folate	0.0004	0.001	0.001
Selenium	0.000019	0.00004	0.00004
Vitamin B6	0.00153	0.00172	0.00172
Vitamin B12	0.00005	0.00012	0.00012
Magnesium	0.05	0.05	0.05
Zinc	0.0012	0.0012	0.0012
<i>Fat source:</i>	5 g soybean oil	2.5 g soybean oil 2.15 g marinol C45 0.35 g ropufa 50	2.5 g soybean oil 2.15 g marinol C45 0.35 g ropufa 50
EPA	0	0.589	0.589
DHA	0	0.382	0.382
AA	0	0.118	0.118
GLA	0	0.0115	0.0115
Total Ω -3	0.155	1.264	1.31
Ω -6/ Ω -3 weight ratio	4.35	1.17	1.137
Linoleic acid	0.640	1.321	1.661
α -linolenic acid	0.155	0.137	0.184
<i>Phospholipids:</i>			
Phosphatidylcholine	0	0	0.2
phosphatidylserine	0	0	0.2

<i>Other components:</i>			
Vitamin C	0	0.2	0.2
Vitamin E	0.00603	0.3	0.3
Coenzyme Q10	0	0	0.03
β -carotene	0	0.02	0.02
flavonoids	0	0.2	0.2
acetylcarnitine	0	0	0.6
choline	0.15	0.15	0.4
thiamin	0.0019	0.0019	0.2
tyrosine	0.944	0.944	1
tryptophan	0.232	0.232	1

2VO surgery

At the age of 4 months the common carotid arteries of half of the animals ($n = 10$) of each dietary group were bilaterally and permanently occluded to induce cerebral hypoperfusion. The other 10 animals per dietary group received the same surgical procedure but the actual occlusion of the arteries was not performed (so called SHAM animals). The surgical procedure was similar to the one reported in the aforementioned paper by de Jong et al. Briefly, the animals were anaesthetized by isoflurane gas. Via a longitudinal, cervical cut on the ventral surface of the neck, the left and the right carotid arteries were located by separating the muscle layers lateral to the trachea. The arteries were carefully separated from the vagal nerve, surgical threads were placed around them and tied up to create permanent occlusions. The wound was closed and the animals were observed during the first week of recovery. A week after surgery the overall survival rate was 88.3 %.

Results

Vascular parameters:

The effect of the 2VO method and the diets on the blood vessel health was studied in terms of the number of degenerating pericytes, i.e. cells that control the blood brain barrier function in the blood vessel walls, the number of endothelial mitochondria and the number of capillaries/ mm^3 after anesthesia of the animals at the age of 7 months. The results hereof are shown in Figure 1, 2 and 3, respectively. In each of these figures

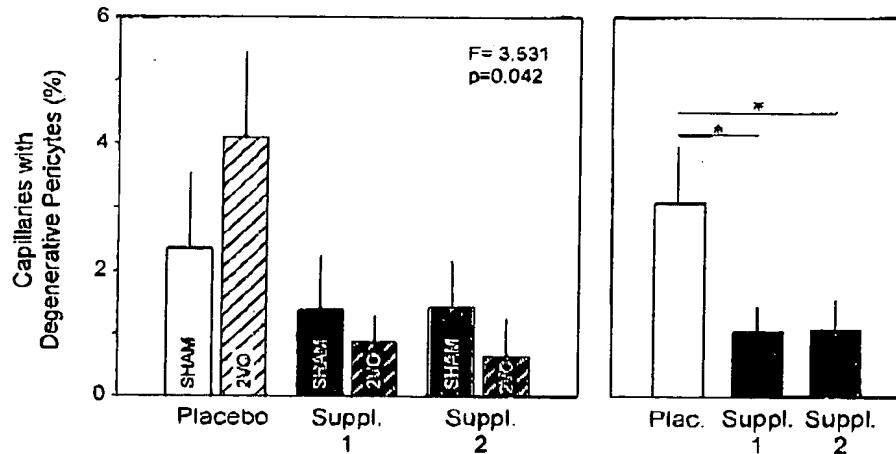
panel B summarizes the differences with diet in all SHAM and 2VO animals.

The right panels of the graphs represent dietary effects of a two-way ANOVA analysis, where 2VO and SHAM animals of the same dietary group are combined. With "ANOVA" it is understood ANalysis Of VAriance between groups.

According to Figure 1 the number of degenerated pericytes was significantly increased following the 2VO procedure. Both supplements effectively reduced the pericytic and thus blood vessel degeneration. Figure 2 demonstrated that both supplements show a decrease of the number of endothelial mitochondria in comparision to 2VO, although the effect is more pronounced in the case of administration of supplement 1 (Fig. 2). (The reduction in mitochondria following supplement 1 and 2 suggests that the lower oxygen supply to the brain induced by 2VO resulting in more activity to maintain energy status is effectively normalized by both supplements.) Figure 3 demonstrated that the density of capillaries in the brain was slightly decreased by 2VO, normalized with supplement 1 and even slightly increased giving supplement 2 (Fig. 3). The amount of capillaries is known to positively correlate with spatial memory (see de Jong et al.). From the blood vessel health experiments it can thus be concluded that the supplements have a beneficial effect, especially supplement 2 (in accordance with the invention).

Pericytic Degeneration

Figure 1



Number of Mitochondria

Figure 2

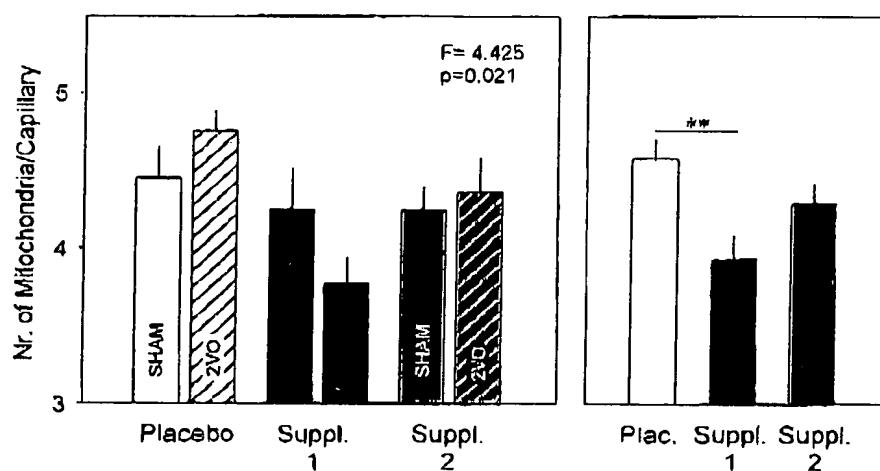
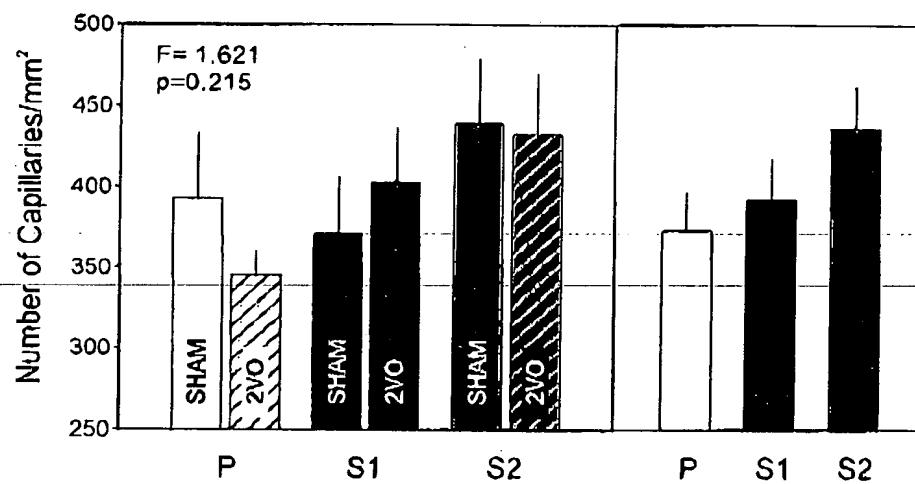


Figure 3



Spatial learning

In a Morris Water Maze (MM) behavioural test the spatial memory performance following 2VO or SHAM procedure with or without diet was studied. In this behavioural paradigm the animals are trained to find a hidden platform in a swimming basin following orientation on specific spatial cues provided by the investigator.

Thereto, at the age of seven months, 13 weeks after surgery, the animals were tested in a Morris water maze consisting of a black, circular water tank (140 cm in diameter) and a square, hidden platform (20.5 cm by 20.5 cm, \pm 1 cm beneath water level). The water was 30 cm deep and had a temperature of 26 ± 1 °C. The water tank was located in an experimental room with various distal cues. The platform was always positioned 35 cm out of the rim of the pool on the same location with respect to the distal, visual cues. A

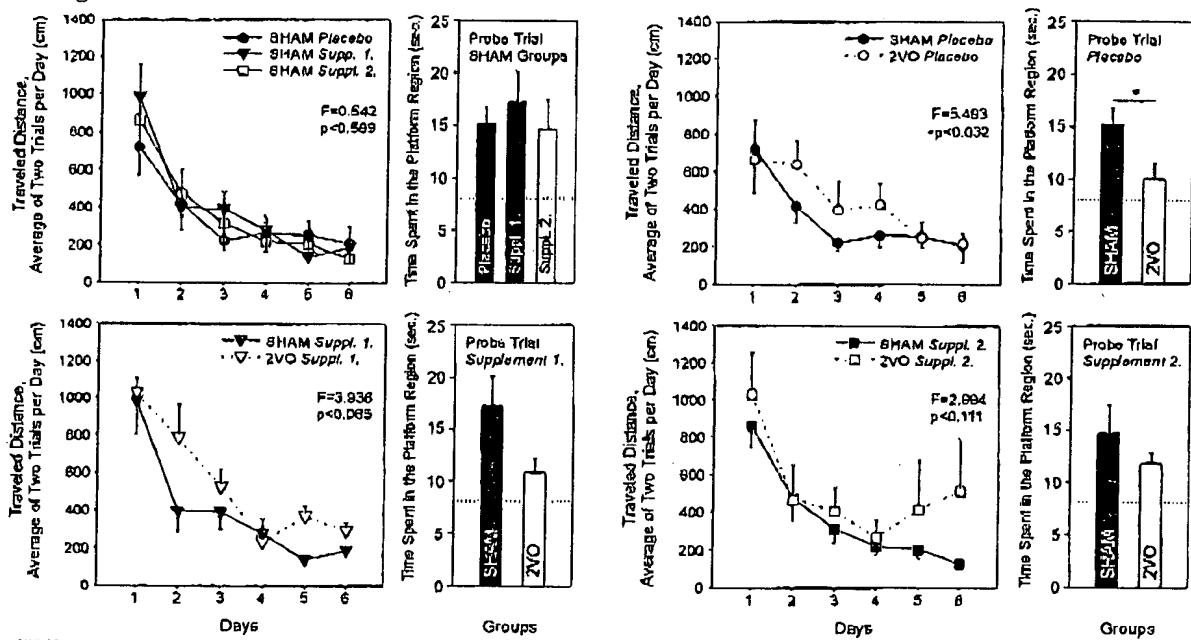
video camera placed 2 meters above the center of the pool was used to observe the animals' swimming pattern. The camera's signal was directed to a computer equipped with videotracking software (EthoVision 2.0, Noldus, Wageningen, The Netherlands). The software was used to divide the water maze into different zones. A platform area was defined as a circle directly surrounding the platform (48.5 cm in diameter). The rest of the pool was considered as the non-platform area. Swimming patterns were analyzed for the time necessary to locate the platform and the distance the animals had traveled before they reached the platform.

The animals were tested two times per day at the end of their activity period and start of their resting period, with a 3 hours interval. There were four different starting point in the water maze: two adjacent to the platform location and two opposite to it. For the first daily trial the animals were placed in the water at one of the close starting points and for the second trial per day they started at one of the far points. The animals were placed in the pool facing the rim and were given 2 minutes to locate the platform. When the rats could not find the platform within 2 minutes, they were gently directed to it. All animals were allowed to sit on the platform for 15 seconds before they were returned to their home cage. The acquisition phase of the test consisted of 5 days with 2 trials per day, where the average swimming distance of the 2 trials was calculated as an indicator of daily performance. Twentyfour hours after the last acquisition trial the animals underwent a probe trial, where the platform was removed from the pool. The rats were allowed to swim for 1 minute and the time spent in the platform area was registered as a measure for retention of the platform location.

The results are shown in Figure 4. When comparing 2VO and SHAM animals in the control dietary group (Fig. 4 upper right panel), 2VO animals performed worse in the learning phase: The animals showed a significant increase in the distance travelled to the hidden platform following 2VO. The diets did not affect MM performance in any of the SHAM groups (Fig. 4, upper left panel). Supplement 1 (Fig. 4, lower left panel) shows no effect either, whereas a significantly improved MM performance was observed for supplement 2 (Fig. 4, lower right panel) after 5 days. Supplement 2 including PUFA's and PL's significantly improves the spatial learning ability of the

animals.

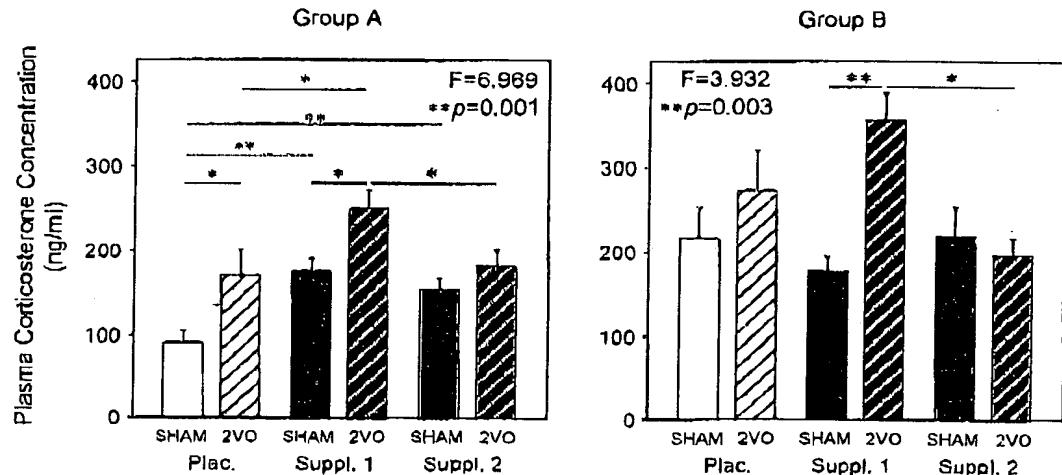
Figure 4



Hypothalamus-pituitary-adrenal axis activity

The effects of 2VO and diet on plasma corticosterone levels at the time of sacrifice of the animals were measured by radio immuno assay. The increase of the corticosterone concentration is a direct stress indicator.

Figure 5



The results plotted in Figure 5 (in which $*p < 0.01$) show that the activity of the stress

axis as reflected by plasma corticosterone levels was significantly increased in 2VO animals on control diet and supplement 1, but not following supplement 2.

Conclusion

These results show that EFA's or BFA's together with PUFA's and extra vitamins (supplement 1) do not improve vascular function to normal in an animal model for atherosclerosis. The combination of polyunsaturated fatty acids, vitamins AND phospholipids according to the invention (supplement 2) provides distinctly different results from supplement 1 and the placebo diet.

Moreover, the experimental results show that supplement 2 yields better blood vessel health and improved spatial memory. Thus, these results give proof of synergistic actions of these diet components on vascular dementia and/or Alzheimer.

Thus, in view of the unexpected results exhibited by the claimed invention and deficiencies of the cited publications, I declare that one skilled in the art would not find that any of the proposed combinations render obvious the claimed invention.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: Wageningen September 15th, 2004
(place) (date)

Signature:

Malvina

Martijn C. de Wilde

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